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13. ABSTRACT (Maximum 200 words)

We performed a series of FTIR measurements of DNA absorption spectra in THz range and confirmed that these spectra of nucleaic acids can serve as a signature of their structure. We also have suggested a method for the prediction of far Infrared absorption spectra in THz region for biomolecules. This theoretical method includes 1) energy minimization, 2) calculation of normal modes, 3) calculation of dipole oscillations, 4) calculations of IR absorption. We have calculated normal modes and their oscillator strengths for a number of synthetic DNA and RNA molecules with known base pair sequence. The calculated positions of the resonance peaks in absorption spectra were found to be in a good agreement with those obtained experimentally, which validates both the predictive power of our theoretical methods and accuracy of experimatal measurements.

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TABLE OF CONTENTS

STATEMENT OF THE PROBLEM STUDIED

DNA low-frequency internal vibrations play an important role in many biological processes, such as transcription, viral infection and molecular recognition. DNA absorption measurements coupled with computations demonstrated that DNA low-frequency vibrations produce characteristic absorption bands in the far IR region (between 40 and 300 cm^{-1}) which are sensitive both to DNA composition and to environment. In the present project we refined the technique for DNA and RNA spectral measurements in submillimeter region and developed a new theoretical method for the prediction of absorption spectra. Thus, we developed new tools which allow us to use charachteristic biomolecular spectra as a signature of their chemical and topological structure.

SUMMARY OF THE MOST IMPORTANT RESULTS

A. Absorption coefficient spectrum

An absorption coefficient spectrum of a Herring DNA film over an extended frequency range is presented in Figure 1. Above about 300 cm^{-1} the absorption spectra consist of numerous sharp, well defined resonances (regions I and II). They represent different covalent short-range, high-energy interactions that have been relatively well studied primarily by Raman spectroscopy. For example, it was demonstrated that in the range 1800-1500 cm^{-1}, the spectrum originates from in-plane double-bond vibration of the bases, and in the range 1500-750 cm^{-1} - from backbone sugar vibration modes. These features tend to be independent of the base-pair sequence. The position of peaks here is well reproducible, does not depend on film thickness, and is the same for both salmon and herring DNA. Since there are no visible interference effects, equation (1) has been used for absorption coefficient calculations in these regions.

For this study we are interested in region III. The absorption coefficient is low in this range and eventually drops dramatically at frequencies below 10 cm^{-1} (Figure 2). This very far IR frequency range has not been widely investigated because of the many challenges for both experimental and theoretical researchers. Also, the interpretation of observed features is not straightforward. For these reasons, we focus our efforts on measuring highly resolved and reproducible spectra in this range. A dip in the absorption coefficient spectrum is observed at about 300 cm^{-1}. This is the short wavelength edge of absorption due to low energy vibrations (weak bonds and non-bonded interactions in DNA). This edge was previously predicted by a theoretical analysis.

In this spectral range we also observe weak features, basically shoulders, that are more easily detected in the derivative of transmission spectra (See figure 4). Earlier, spectral features observed in the spectra of nucleic acids in the region 300-500 cm^{-1} were assigned to the ribose ring vibrations . The differential plots reveal a large number of weak shoulder features in the transmission characteristics over the range 300-500 cm^{-1}. These features are actually soft phonon modes that are obscured by the absorption roll-off from the very strong resonance at 545 cm^{-1}. The experimental results from the 300 - 500 cm^{-1} range show that all clearly-resolvable phonon modes are present in both salmon and herring DNA samples. The position of the peaks in the derivative of transmission spectra (i.e., dT/df, where f is a frequency, calculated from smoothing over 9 data points) are the same for herring and salmon DNA and does not depend on the thickness. Figure 4 compares the differential spectral results from herring DNA films prepared with a 10:1 and a 5:1 water-to-DNA concentrations. Clearly, there is significantly stronger coupling of the electromagnetic energy when the samples are prepared with larger concentrations of water. While the fundamental mechanism responsible have not been identified at this point, it is clear that the oscillator strength (i.e., either the phonon density or polarizability) has been affected by the hydration level. The structures disappear with reducing the amount of water thus indicating that corresponding vibrational modes occur at the interface between DNA and surrounding media. Understanding this phenomenon will have many important ramifications to resolve the dynamics within intrinsic (isolated) DNA macromolecules. For example, if this effect is related to salinity of the sample (e.g.,

the DNA samples under study are in fact DNA salts) then the prescription for enhancing the phonon activity is important for interrogating the microscopic physical dynamics.

The broad peak in the long-range absorption spectra as presented in Figure 3 agree qualitatively with earlier studies performed by Powell et. al. on vacuum-dried poly(dG) · poly(dC) DNA (G-guanine, C-cytosine).

B. Low frequency resonanses in transmission

The basic findings and most important aspects of this research are now presented. In this work, the focus was on receiving highly resolved and reproducible transmission spectra in the lower end of the far IR region which is the most difficult to realize, where reliable data were practically absent, and where specific features in spectra of different biological objects were expected to be found. An extensive series of measurements carried out in the very far IR frequency region with a higher resolution of 0.2 cm^{-1} revealed fine features in the spectra which can be more or less pronounced depending on the quality of material and sample preparation and measurement conditions. The transmission spectra of calf thymus DNA demonstrating this fine structure are given in Figure 1, as do the salmon and herring DNAs in Figure 3, the artificial polynucleotides, poly-adenylic acid potassium salt (Poly [A] RNA) in Figure 4. Although the observed amplitude of the resonant modes is not greater than several percent, the signal-to-noise ratio of the instrument is good enough to detect these features in spectra of free standing films, as well as of films on polycarbonate or Teflon substrates. This fine structure was found to be independent of sample-preparation and water content. The fine structure observed here requires spectral resolution better than 0.5 cm^{-1}, which is difficult to achieve because of very low source energy in the submillimeter range. This requirement may partially explain why earlier FTIR investigations were not able to detect phonon resonances. Peaks appear with a density of approximately one per cm^{-1} in the interval between 10 and 200 cm^{-1}. This general density of frequencies in this regime had been predicted earlier. The intensity of absorption lines gradually drops towards the upper end of the band. Here, the aperture of the spectrometer determined the lower-frequency limit.

4

As far as we know, this is the first demonstration of submillimeter-wave spectroscopic features that can serve as DNA signatures.
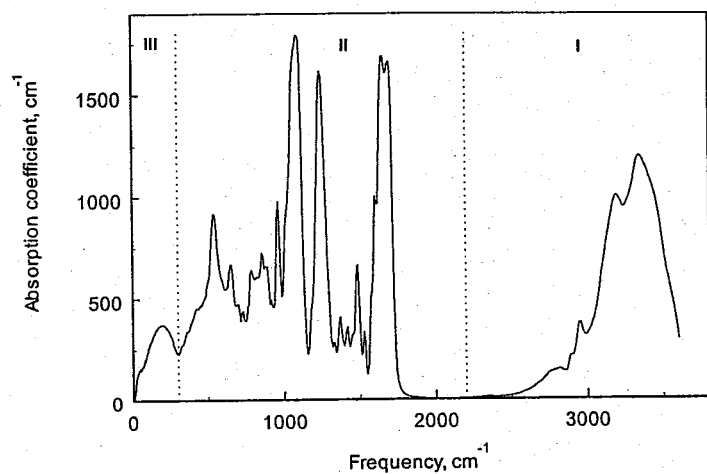

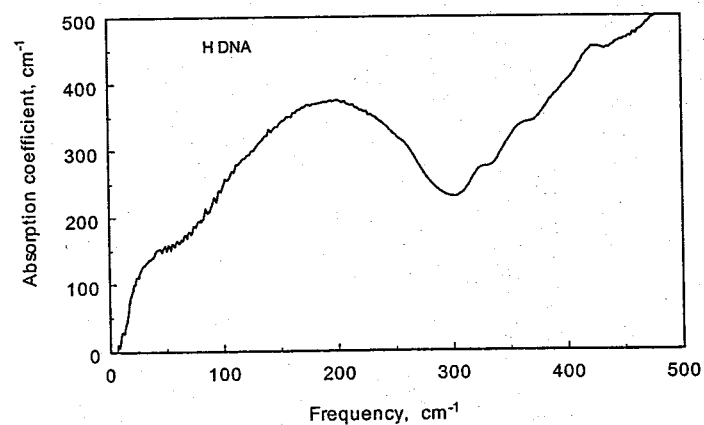Figure 1. Absorption spectrum of herring DNA in the extended energy range.

Figure 2. Absorption spectrum of herring DNA in the range 10- 500 cm^{-1}.
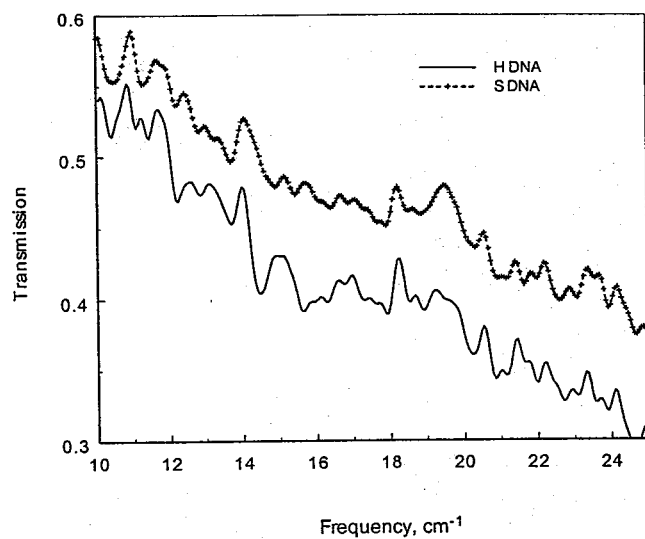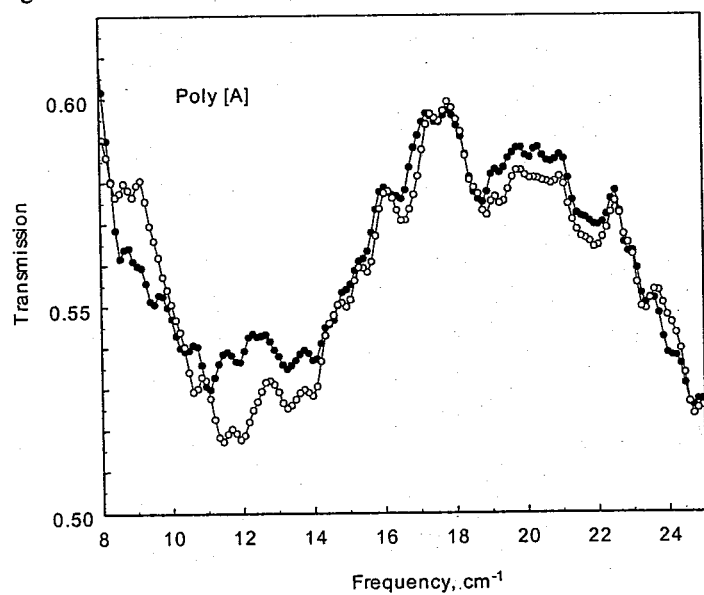
Figure 3. Fine structure in transmission spectra of herring (H) DNA and salmon (S) DNA in the range 10-25 cm^{-1}.

Figure 4. Fine structure in spectra of Poly [A] RNA. Two independent measurement results.

C. *Theoretical prediction of absorption spectra*

The dependence of permittivity ε on radiation frequency ω can be described by the

Kramers – Heisenberg dielectric function:

$$\varepsilon(\omega)=\varepsilon_\infty + 4\pi N \sum_k S_k/3(\omega_k^2-\omega^2 - i\,\gamma_k\,\omega/2\pi\;)\;, \qquad (1)$$

where index k denotes normal modes, ω_k are vibrational frequencies, S_k are oscillator strengths,

γ_k are oscillators dissipations, and N is the number of molecules.

Absorption coefficient (α) is proportional to the imaginary part of permittivity:

$$\alpha= (\omega/nc)\,\mathrm{Im}\,(\varepsilon\,), \qquad (2)$$

hence its dependence on frequency ν ($\nu=\omega/2\pi$) is determined by:

$$\alpha(\nu)\sim \nu^2 \sum_k S_k\gamma_k / (\,(\nu_k^2-\nu^2)^2 + (\gamma_k\,\nu)^2\,) \qquad (3)$$

Normal mode analysis enables calculations of normal frequencies ν_k and oscillator strengths S_k, while

oscillator dissipations γ_k remain to be determined from the comparison of theory and experiment. In the

first approximation, however, the decay can be considered frequency independent. In this simplified

form the absorption will depend on the frequency as:

7

$$\alpha(\nu) \sim \nu^2 \gamma \sum_k S_k / ((\nu_k^2 - \nu^2)^2 + (\gamma\nu)^2) \qquad (4)$$

The realistic estimation of γ obtained from the measurements of (poly dA)(poly dT) absorption spectra is from 2 to 7 cm^{-1}.

Standard B-helical conformations were created by the program package JUMNA. Conformational energy was calculated as a sum of an energy of van der Waals and electrostatic interactions, torsion rotation potentials, harmonic potentials of bond angle deformations, and the energy of hydrogen bonds deformations using FLEX force filed. The energy was minimized in the space of internal coordinates of a molecule (torsion and bond angles) using software the package LIGAND.

Normal modes (eigenfrequencies **W** and eigenvectors **A**) were calculated in the space of internal variables as solutions of the equation:

$$\mathbf{HAW = FA} \qquad (5)$$

where W is a diagonal matrix with elements ω_k^2 (vibrational frequencies); **F** is a matrix of second derivatives of potential energy and **H** is a matrix of second derivatives of kinetic energy. The normal modes (matrixes **A** and **W**) were calculated using the program LIGAND.

Each normal vibration produces fluctuations of the dipole moment of a molecule. Oscillator strength S_k corresponding to k^{th} normal mode can be expressed as a square of normalized amplitude **(p)** of the dipole moment deviation :

$$S_k = (\mathbf{p}^k)^2 \qquad (6)$$

The normalized dipole moment **p** can be expressed as:

—

8

$$p = \sum_i e_i \, \mathbf{a}_i / \sqrt{m_i} \qquad\qquad (7)$$

where index i denotes atoms, e_i are partial atomic charges, m_i are atomic masses, and $\mathbf{a}_i$ are eigenvectors (atomic displacements) normalized in such a way that:

$$\sum_i (\mathbf{a}_i)^2 = 1 \qquad\qquad (8)$$

The procedure consists of :

1) Initial optimization of the B-helix by the program JUMNA;

2) Energy minimization and calculation of normal modes (eigenfrequencies ω_k and eigenvectors $\mathbf{A}_k$) by the program LIGAND.

3) Calculation of three dimensional structures of a molecule along normal vibrations [7].

4) Calculation of oscillator strengths. The variable dipole moment of a molecule (**P**) is calculated as:

$$P = \sum e_i \, \mathbf{r}_i \qquad\qquad (9)$$

Where the index i denotes atoms and the vector $\mathbf{r}_i$ is a displacement of ith atom from equilibrium. Oscillator strengths S_k are calculated as:

$$S_k = (P^k)^2_{max} / \sum m_i \, (\mathbf{r}_i)^2 \qquad\qquad (10)$$

where P^k is produced by k^{th} vibration.

5) Calculation of absorption spectra $\alpha(\nu)$ (Eq. 4).

Oligonucleotides (Poly dA)$_{12}$(Poly dT)$_{12}$ and (Poly dAdT)$_6$ (Poly dTdA)$_6$ have 360 degrees of freedom in the space of internal coordinates of a molecule (torsion and bond angles). Respectively, 360 normal modes were found for each sequence. Their frequencies lie below 900

cm-1, so there is almost no overlap with vibrations of covalent bonds which have frequencies above 750 cm^{-1}. The two oligonucleotides have similar mode densities in the region above 250 cm^{-1}, which essentially involve vibrations of valence angles. It could be expected that the modes that reflect vibrations of torsion angles would be more sensitive to the conformation and flexibility of the helix. Indeed, the calculated density spectra of the two oligos differ markedly in the region below 250 cm^{-1}. Specifically, (Poly dAdT)$_6$ (Poly dTdA)$_6$ has very distinct peaks around 15 and 175 cm^{-1}, while homoplymer (Poly dA)(Poly dT) with unique conformational properties [14] has a broad spectrum with no pronounced peaks (Fig. 1). The earlier normal mode analysis of (Poly dAdT)$_6$ (Poly dTdA)$_6$ performed with rigid valence angles revealed only one peak at 15 cm^{-1}, which indicates that the second peak at 175 cm^{-1} essentially involves vibrations of valence angles.

Both Poly (dA)Poly(dT) and Poly (dAdT)Poly(dTdA) have a resonance absorption at the frequency 60 cm^{-1}, therefore we examined deviations of a dipole moment P^k (Eq. 7) produced by the strongest mode with the frequency near 60 cm^{-1}. This mode was previously assigned to vibrations along the helix axis (Z), however, Fig. 2 demonstrates that the traverse component P_x has comparable amplitude.

The amplitude of P^2 fluctuations determines the strength of each vibration (Eq. 9). Normal modes of the two examined oligos demonstrated very different distributions of oscillator strengths. As would be expected, for the molecule with homogeneous strands Poly(dA)Poly(dT) the oscillator strengths appeared to be very uniform, while heterogenity in the strands composition introduces many-fold variance. The two lowest-frequency modes (2.1 and 2.6 cm^{-1}) have strengths 3-4 times greater than the average strength for the other modes. These two lowest-frequency vibrations are have strong P components perpendicular to the helical axe, and they

apparently reflect helical bending. The strongest modes of the heteropolymer (Fig. 3 A) also lie in the lowest frequency range (mostly below 50 cm^{-1}). This result is not surprising, for the strongest modes reflect the relative motions of the strands, which in turn involve low-frequency torsion rotations. Correspondingly, the normal modes spectrum weighted by oscillator strengths is essentially shifted towards low-frequencies for the heteropolymer, while for the homopolymer heterogenity in oscillator strengths has a minor effect compared to the normal mode density itself.

We calculated absorption spectra of the two molecules (Fig.5) according to Eq. 4 using two reasonable approximations for the band width γ obtained experimentally (2 and 7 cm^{-1},). The positions of resonance peaks do not depend on the band width (Fig. 5), and in the longer wave range (below 220 cm^{-1}) they are remarkably similar to those observed experimentally. Another similarity of the calculated and experimental spectra is the steep fall in absorption intensities as the frequency exceeds 250 cm^{-1} for both sequences. This feature is caused by the decrease in normal mode densities and is not related to their optical properties. Finally, Poly(dAdT)Poly(dTdA) demonstrates higher absorption at lower frequencies (below 100 cm^{-1}) due to the higher oscillator strengths of its lower frequency modes, while Poly(dA)Poly(dT) has a plateau between 20 and 200 cm^{-1}. This difference in the spectra has also been observed experimentally.

In spite of many factors, such as variability of molecule length and interaction with salt and water molecules, the absorption peaks calculated in our study for Poly(dA)Ploly(dT) reproduced most of the peaks observed in FIR spectroscopy. Thus, the presented analysis has a considerable potential for the prediction of DNA optical properties.
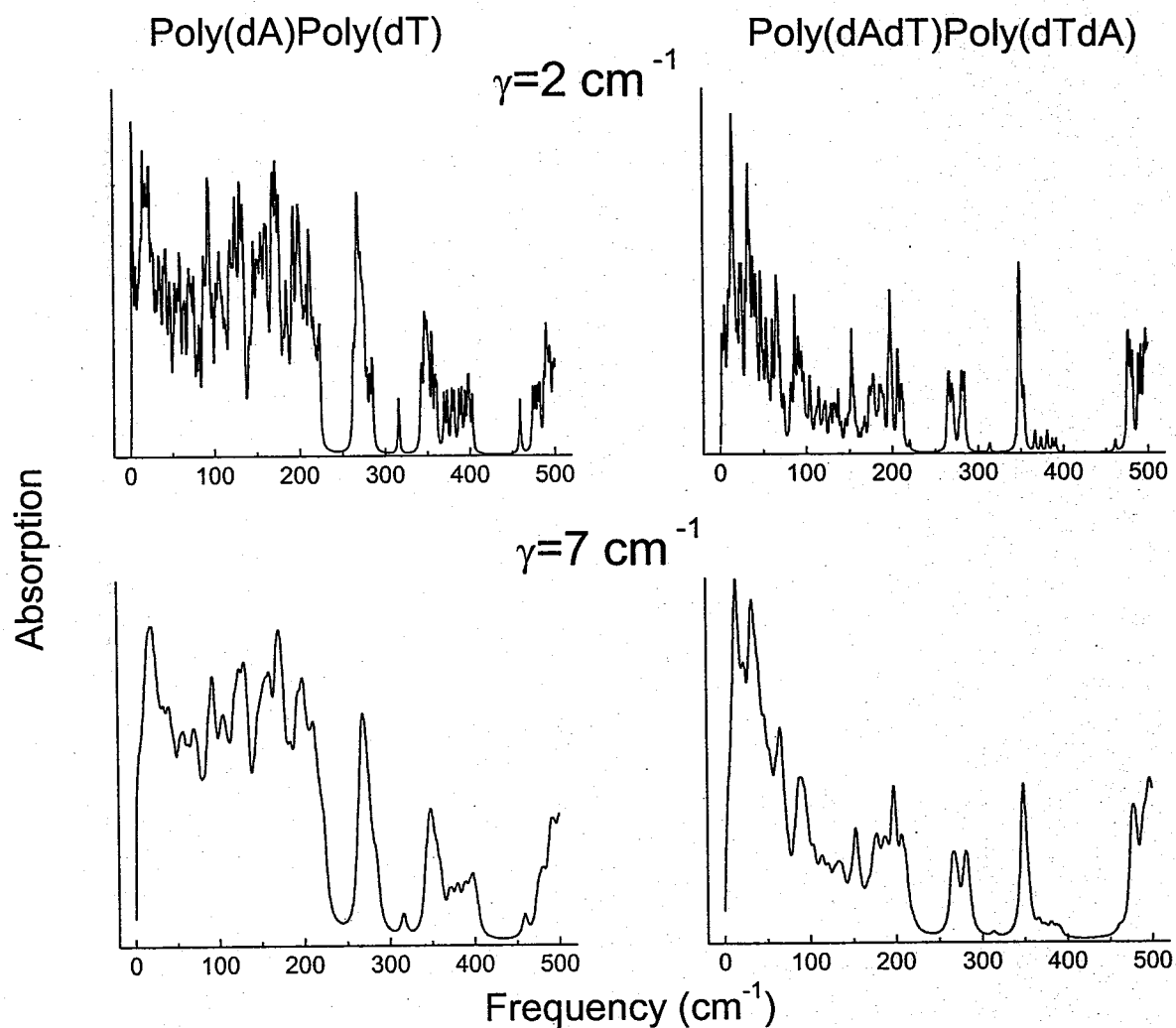
Fig. 5. Absorption spectra (arbitrary units) of Poly(dA)$_{12}$Poly(dT)$_{12}$ (left) and (Poly dAdT)$_6$ (Poly dTdA)$_6$ (right) calculated at two values (2 and 7 cm^{-1}) of the band width γ.

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